Preliminary Investigation using a Batch Flow Process to Determine Bacteria Destruction by Microwave Energy at Low Temperature

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Introduction

Food is preserved in many ways — canning, freezing, drying, salting, pickling, etc. The food industry is always searching for better ways of preservation to improve product quality that employ less heat, mechanical shear, and additives.

Most foods absorb energy from high frequency electromagnetic fields, such as microwave energy. 'Electromagnetic field effects ... arise from interactions between the electrical components of an electromagnetic field ... and the chemical constituents of foods. These interactions result primarily from the dipole rotation of free water molecules and from the conductive migration of charge carriers...' (1). There is some debate as to whether there are nonthermal effects associated with microwave processing which could be used to destroy bacteria without heat generation. For example, Mudgett (2) found no nonthermal effect, i.e. 'Microbial survival and thermal denaturation of heat labile constituents in microwave processing are governed by the same time–temperature relationships as those in conduction heating'. Goldblith and Wang (3) detected no difference when suspensions of *Escherichia coli* and *Bacillus subtilis* spores were exposed to both conventional and microwave heating. Welt *et al.* (4) investigated the effect of sublethal temperature treatment with microwave energy on *Clostridium sporogenes* in potassium phosphate buffer and found no additional lethality aside from the rapid heating offered by microwave radiation.

On the other hand, Webb and Dodds (5, 6) reported cells of *E. coli* grown in nutrient broth and exposed to microwaves exhibited slower cell division and inhibited metabolic processes early in the life span of the cell. Culkin and Fung (7) studied the effect of microwaves on *E. coli*, and *Salmonella typhimurium* in tomato soup, vegetable soup, and broth. Their 'data suggest that the heat generated during the microwave exposure alone is inadequate to fully account for the nature of the lethal effects of microwaves for microorganisms'. Olsen (8) observed that the numbers of viable spores of *Aspergillus niger, Penicillum* sp. and *Rhizopus nigricans* were greatly reduced by microwaves during bread-making.

Several theories have been advanced to explain how electromagnetic energy might kill microorganisms without heat. These theories are summarized in a review by Knorr *et al.* (9). There is the 'dielectric rupture theory' of Zimmermann *et al.* (10) in which an external electric field induces an additional transmembrane electrical potential which is larger than the normal potential of the cell. At sufficient potential the cell membrane ruptures, resulting in pore formation, increased permeability, and loss of cell integrity. Kinosita and Tsong presented a mechanism for pore development caused by pulsating electric fields (11). They present evidence of migration of intracellular solutes across the cell membrane and resultant destruction of the cell.

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Another possible explanation for the nonthermal effects observed intermittently when employing electromagnetic energy fields is the interaction of the associated magnetic field with the microorganisms. In a review by Pothakamury *et al.* (12) they conclude that the 'inhibitory effect of static or oscillating magnetic fields on the growth of microorganisms exhibits the potential to inactivate microorganisms in food'.

Mertens and Knorr (13) discuss the use of oscillating magnetic fields disclosed in a world patent (14). 'The patent suggests that the oscillating magnetic field couples energy into the magneto-active parts of large biological molecules with several oscillations. When a large number of magnetic dipoles are present in one molecule, enough energy can be transferred to the molecule to break a covalent bond. It is assumed that certain critical molecules in a microorganism, like DNA, or proteins, could be broken by the treatment, hence destroying the microorganism or at least rendering it reproductively inactive'. The goal of our research is to assess the usefulness of microwave energy to kill bacteria in liquid foods at reduced temperatures and to develop a process which minimizes thermal damage. This particular phase of the research addresses the effect of microwave energy on *Pediococcus* sp. in a batch (full recycle) flow system.

Experimental

Our experimental process combines instant energy input to the food system using electromagnetic energy (EME), such as microwave, with rapid removal of thermal energy utilizing an efficient heat exchanger mechanism. In the prototype system, a double tube heat exchanger inside a continuous microwave dryer was used. The outer tube was 33.8 mm i.d. polypropylene which is transparent to EME. The inner tube was 25.4 mm o.d. stainless steel. Cooling water flowed through the inner tube. Nineteen litres of process fluid circulated counter-current through the annulus. The volume of the annulus within the microwave chamber was 1.34 L. The total volume of the system, excluding the feed tank, was 15.75 L. The EME energized the process fluid but was reflected off the inner tube. The annulus is an energy trap and the inner tube is a heat sink. The inner tube removed energy from the process fluid to prevent a significant temperature rise. The effect is rapid destruction of bacteria with virtually no thermal degradation of the food.

Figure 1 shows the experimental set up. The feed tank was 190 L stainless steel. Feed solution was inoculated with *Pediococcus* sp. NRRL B-2354 (formerly *Micrococcus freudenreichii*) to achieve a nominal bacterial count of log 6.5 cfu/mL. *Pedicoccus* sp. was originally inoculated by transferring 2.5–5.0 mL of stock solution into 250–1000 mL TGY broth (5 g tryptone, 1 g glucose, 5 g yeast extract, 1 g K_2HPO_4, 1 L distilled water) and incubating at 37 °C for 18–24 h. The final count was 8.0–9.0 log.

A sanitary lobe pump, TriClover rotary pump (Kenosha, WI), model #PRED3-1M-UC6-ST-S, controlled the process fluid flow rate at 0.77 to 3.4 kg/min. The process fluid went to a 7 kW continuous microwave dryer (Cober Electronics Inc., Stamford, CT). It passed through the microwave in the annulus of a double pipe heat exchanger. The outer pipe, which contained the process fluid, was Sanitech -T1-1/2″ sanitary pipe which was 33.8 mm inner diameter and made of polypropylene. The inner pipe was stainless steel with a 25.4 mm outer diameter and 1.7 mm wall thickness. Tap water was used as the cooling fluid in the inner pipe. Leaving the microwave, the process fluid went through a plate and frame heat exchanger (DeLaval, Sweden, model P5VER). Tap water was used as the cooling fluid in the heat exchanger which then went to the microwave cooling tube. Cooling water was at a nominal 11.3 kg/min at 20–25 °C. After the heat exchanger, the process fluid returned to the feed tank. Samples were taken in the recycle line to the feed tank. Samples were taken in triplicate and plated on TA plates (tryptose agar) and incubated at 37 °C for 18–24 h. Counts were retrieved using a bacterial colony counter (model 500A, Spiral System Instruments, Inc, Cincinnati, OH). A thermocouple was placed at the processing fluid exit of the microwave. A fiber optic probe, Luxtron Fluoroptic Thermometer (Luxtron Corp., Santa Clara, CA, model 950) was inserted into the process tube to measure the temperature distribution of the process fluid within the microwave. At normal steady state operating conditions, the probe was moved slowly within the tube over the length of the heating section (under the wave guides). **Figure 2** shows the temperature distribution and the corresponding exit temperature. Generally, the thermocouple agreed with the fiber optic probe. With the cooling tube on and under normal experimental conditions, the process fluid temperature was usually about 35 °C with a hot spot at about 40 °C.

In a few runs a temperature indicator label, Tempilabel (Tempil Division, Big three Industries, Inc., South Plainfield, NJ), was placed in the microwave heating section. The label had an irreversible color indicator for 60 °C. With the cooling tube on and under normal

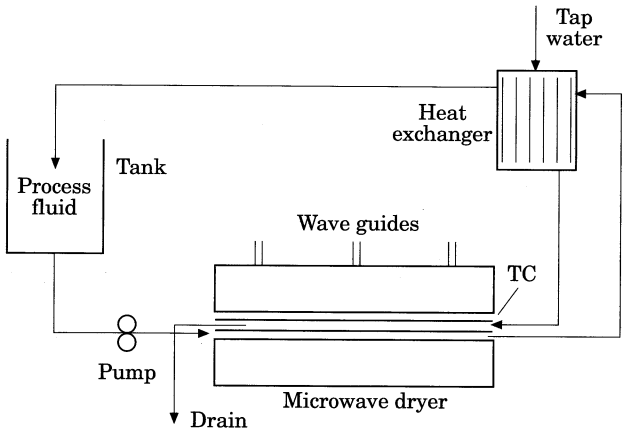

Fig. 1 Flow sheet of the experimental process. TC indicates the location of the exit thermocouple

experimental conditions, the indicator did not change color, confirming that the process fluid did not reach 60 °C.

A residence time distribution study of the complete process was made at a flow rate of 1.43 kg/min. Samples were taken from the recycle line entering the feed tank. The process was started with tap water and then switched to brine. As shown in **Fig. 3**, the process, excluding the feed tank, is about 90% plug flow (15).

In each experiment, 19 L of feed was charged to the feed tank and inoculated. This charge was circulated through the microwave and plate and frame heat exchanger for 1 to 2 h to achieve a uniform distribution. The feed was sampled and the microwave turned on at 5–5.4 kW. Temperature was continuously recorded at the exit from the microwave by computer and effluent from the heat exchanger was sampled periodically for plate counts.

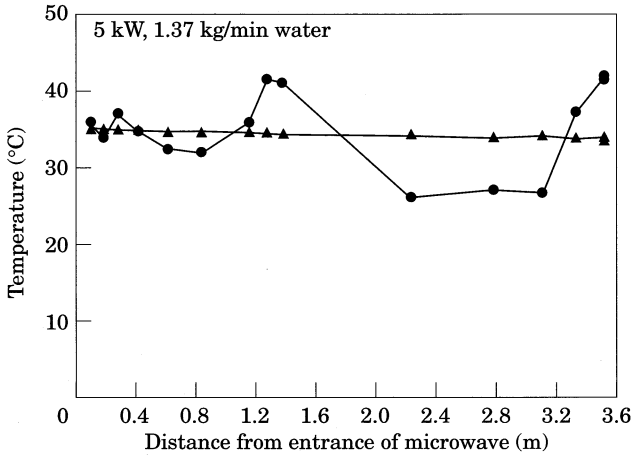

Fig. 2 Temperature distribution of the process fluid in the annulus within the microwave heating zone. The exit thermocouple (▲) location is indicated in **Fig. 1**. The fiber optic probe (●) was slowly pulled through the annulus in the microwave heating zone

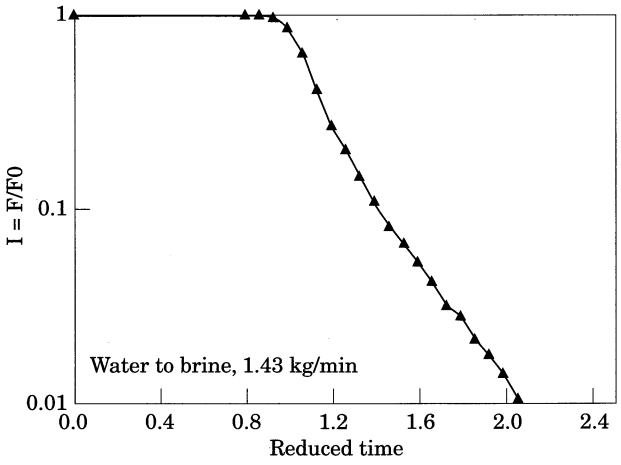

Fig. 3 Residence time distribution through the microwave. Reduced time is the process time divided by the nominal dwell time for one pass. The y-axis is logarithmic internal age distribution function where F is the concentration of the tracer.

Results and Discussion

Figure 4 shows the results when the feed was water. **Table 1** summarizes the experimental parameters and results. There were 3 logarithmic cycle reductions in bacteria after 70 min of processing. The microwave exposure time per pass was 103 s. This was calculated as the liquid volume within the microwave (1.34 kg process fluid/pass) divided by the liquid flow rate (0.78 kg/min).

$$\frac{1.34 \text{ kg process fluid}}{\frac{\text{pass}}{\frac{0.78 \text{ kg}}{\text{min}}}} \times \frac{60 \text{ s}}{\text{min}} = 103 \text{ s/pass}$$

The total microwave exposure time for 19 L was 4.9 min calculated as the number of passes during the experiment (70 min total process time/19 kg/pass/0.78 kg/min) times the time per pass (103 s). The number of passes was determined from the total processing time of the experiment (70 min) divided by the time to process the total fluid volume once in the microwave. The time to process the total fluid volume once was the fluid volume treated (19 kg) divided by the flow rate (0.78 kg/min).

$$\frac{70 \text{ min total process time}}{\frac{19 \text{ kg/pass}}{0.78 \text{ kg/min}}} \times \frac{103 \text{ s}}{\text{pass}} \times \frac{\text{min}}{60 \text{ s}} = 4.9 \text{ min}$$

The temperature remained well below normal temperatures used to destroy bacteria throughout the run. This indicated that microwave energy can destroy bacteria in water with minimum heat treatment.

Fruit juice is approximately 100 g/L sugar plus small amounts of flavor components, pectin, etc. **Figure 5** shows results using 100 g/L sugar solutions (glucose and sucrose) instead of water. The experiments were performed as above except bacteria level was monitored before turning on the microwave. The bacteria were evenly distributed and stable. In the experiment with glucose, the microwave was turned on at 2 h. There

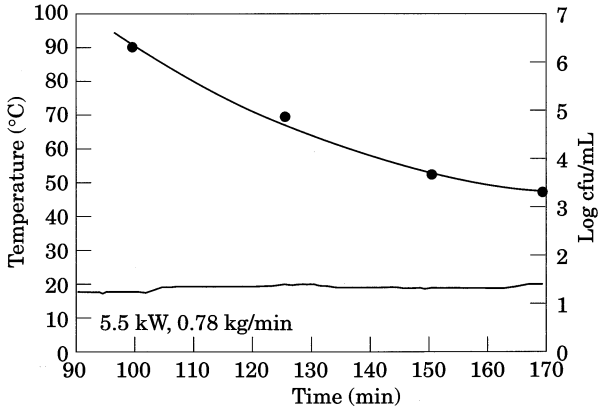

Fig. 4 Microwave treatment at 5 kW of water at low temperature. (●) *Pediococcus* sp. (log cfu/mL); (—) = microwave temperature (°C)

was a 1 log reduction in 20 min of processing. The microwave exposure time per pass was 59 s calculated as explained above:

$$\frac{1.34 \text{ kg process fluid}}{\text{pass}} \times \frac{60 \text{ s}}{\text{min}} = 59 \text{ s/pass}$$
$$\frac{1.37 \text{ kg}}{\text{min}}$$

The total microwave exposure time for 19 L was 1.4 min calculated as explained previously:

$$\frac{20 \text{ min total process time}}{\frac{19 \text{ kg/pass}}{1.37 \text{ kg/min}}} \times \frac{59 \text{ s}}{\text{pass}} \times \frac{\text{min}}{60 \text{ s}} = 1.4 \text{ min}$$

The exit temperature remained below 35 °C.

The experiment was repeated with sucrose. The initial flow rate yielded an exposure time of 24 s per pass but there was no destruction (**Fig. 5**). When the flow rate was reduced so that the exposure time increased to 59 s per pass there was a 3 log reduction with a total microwave exposure time of 4.2 min. Apparently, there is a minimum exposure time needed to achieve destruction.

A similar experiment was also performed with a brine solution of conductivity 9.5 mhos. As shown in **Table 1**, there was a 3 log reduction in cells with a total microwave exposure time of 5.8 min and a 59 s exposure time per pass.

Having established that microwave energy can destroy bacteria in water, brine, and sugar solutions without significantly heating the bulk liquid, the study was extended to apple juice. Apple juice presented an experimental problem of foaming, inherent only to this particular full recycle process. It made it difficult to properly attain a desirable pretreatment bacteria level. However it should cause no problem in future research when the recycle is eliminated. **Figure 6** shows the results for apple juice. The exposure time per pass was 59 s. There were 1 and 2 log reductions in cells with temperatures at or near 35 °C. This indicates microwave energy might be used to destroy bacteria at cold temperatures in apple juice and probably other juices. An improved experimental process will be needed to thoroughly investigate fruit juices and other products.

In these experiments, the bulk liquid did not reach sufficiently high temperatures to account for bacterial destruction. These results seem to confirm the theory (9) that microwave energy can destroy bacteria without heat. However, the results are confounded with possible side effects. Apple juice is acidic, as are most liquid foods, and the acidity may contribute to the effect of the microwaves on the bacteria. Also, the bacteria in the liquid cools after passing through the microwave. This may give the bacteria time to repair and/or regenerate to counter the effects of the microwave. To further investigate the ability of microwave energy to destroy bacteria without heat, a series of three runs was performed (**Fig. 7**). In one run there was no cooling. However, the temperature was maintained at about 35 °C by using a microwave energy setting of 3 kW. There was minimal destruction; 1.5 log reduction in cells after 2.5 h. In a second run microwave energy was applied at 5.4 kW with cooling to maintain the bulk fluid temperature at 35 °C: there was a 3.8 log reduction in cells. In the third run the two above experiments were combined. Initially microwave energy was applied at 3 kW with no cooling but at a bulk fluid temperature of 35 °C. There was no destruction after applying microwave energy for 1 h (experiment time = 85 min). Cooling water was added and the microwave energy raised to 5.4 kW after 125 min experiment time (**Fig. 7**). By adjusting the cooling water rate we maintained the bulk fluid temperature at about 35 °C. As shown in **Fig. 7**, at the same bulk fluid temperature but with higher microwave energy input, the concentration of *Pediococcus* sp. was reduced by 1.2 log. This apparently indicates that microwave energy may destroy bacteria without heat.

Table 1 Microwave treatment results

System	Log kill[a]	Flow rate (kg/min)	Microwave exp/pass (s)	Total microwave exposure (min)	Total process time (min)	q_c[b]	q_p[c]	Δq[d]
Water	2	1.13	24	8.6	120	48	126	138
Water	3	0.78	103	4.9	70	216	3	94
Water	3	0.76	106	4.6	65	–	–	–
Glucose	0.2	3.44	24	4.3	60	131	84	97
Glucose	1	1.37	59	1.4	20	184	10	118
Glucose	2	1.41	57	5.3	75	139	50	53
Sucrose	0.3	3.40	24	4.2	60	–	–	–
Sucrose	3	1.36	59	4.2	60	196	57	59
Brine	0.5	3.11	26	4.3	60	57	135	120
Brine	3.4	1.37	59	5.8	90	49	57	206
Apple juice	1	1.37	59	1.2	60	62	65	196
Apple juice	2	1.37	59	8.5	120	48	66	198
Skim milk	0	0.85	94	4.2	60	–	–	–

[a]Mean value of three determinations.
[b]q_c = energy removed by cooling water (kJ/min).
[c]q_p = thermal energy absorbed by the process fluid (kJ/min).
[d]Δq = difference in energy input (5.2 kW = 312 kJ/min) and output ($q_c + q_p$).

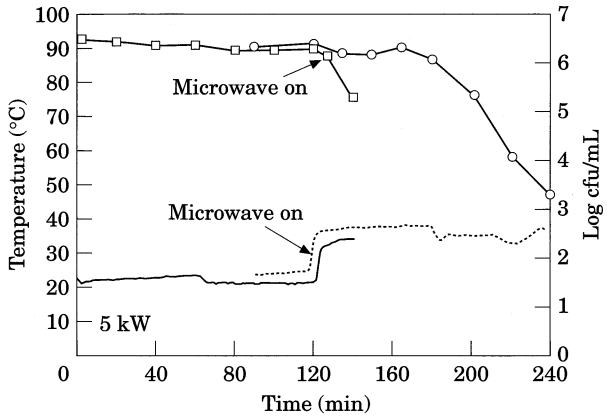

Fig. 5 Microwave treatment at 5 kW of sugar solution at low temperature. (□) = *Pediococcus* sp. in 100 g/L glucose at 1.37 kg/min (log cfu/mL); (○) = *Pediococcus* sp. in 100 g/L sucrose at 3.40 kg/min, change to 1.32 kg/min at 180 min (log cfu/mL); (—, – – –) = microwave temperature (°C)

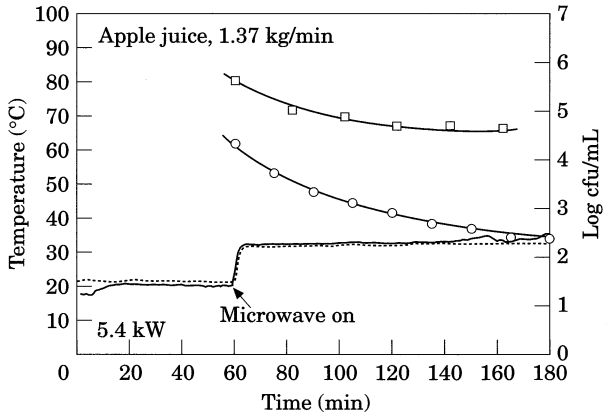

Fig. 6 Microwave treatment at 5.4 kW of apple juice at cold temperature. (○, □) = *Pediococcus* sp., replicate experiments (log cfu/mL); (—, – – –) = microwave temperature (°C)

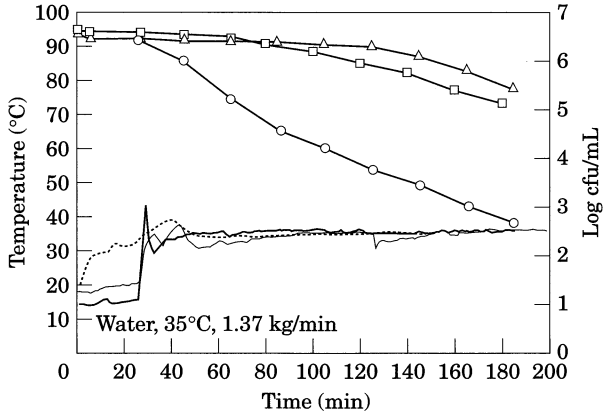

Fig. 7 Comparison of bacterial inactivation (log cfu/mL) in water at two microwave energy levels but same bulk fluid temperature. (○) = 5.4 kW microwave energy setting; (□) = 3 kW microwave energy setting; (△) = 3 kW changed to 5.4 kW microwave energy setting at 125 min. (—, ——, – – –) = microwave temperature (°C)

However, these experiments are still not definitive. An energy balance over the microwave chamber was calculated and **Table 1** lists the energy removed by the cooling water and the energy changed to thermal energy in the process fluid. Assuming 5.2 kW (312 kJ/min) input from the microwave, the difference between 312 kJ/min and the summation of the cooling water and process fluid energy absorption gives the energy available to destroy bacteria. Unfortunately, there is much scatter in the results. In many of the experiments the difference in cooling water temperature was only a few degrees which is within the experimental accuracy of thermocouples. Much more sophisticated controls are needed to determine the exact amount of microwave energy absorbed by the process fluid. At 5–5.4 kW sufficient energy passes through the liquid to raise the temperature to a peak of about 60–80 °C if there was no simultaneous cooling. There is always the possibility of differential heating of the bacteria and/or differential cooling of the fluid food.

We can consider the bacteria as ionic spheres suspended in the fluid. The relative rate of energy absorption depends on the dielectric properties of the fluid and the bacteria. It is possible that bacteria and water have similar dielectric constants; and it is also possible that the bacteria have a higher dielectric loss factor. This would favor enhanced energy absorption by the bacteria and form the basis of differential heating. Differential cooling refers to the greater transfer of absorbed energy from the fluid to the cooling water than from the bacteria. The bacteria transfer the absorbed energy to the surrounding fluid and the surrounding fluid transfers the energy to the cooling water across the stainless steel pipe. If the cell membrane exhibits significant film resistance to energy transfer, the bacteria could be at a higher energy level than the surrounding fluid. However, we know of no noninvasive method to measure the energy content of the bacteria cells. Continued research is planned to determine if bacteria can be destroyed in the fluids without thermal damage. First we plan to develop a continuous, no recycle process. Then to determine the amount of thermal damage to the product by chemical analysis of thermal damage indicators. Organoleptic analysis will form the final evaluation of the extent of thermal damage.

Conclusions

Microwave energy can be manipulated to substantially reduce bacteria, specifically *Pediococcus* sp., in water, brine, or fruit juice without raising the temperature of the bulk liquid to temperatures normally employed to destroy bacteria. This does not require that thermal and microwave effects are completely separated. A cooling tube which passed through the center of the plastic pipe inside the microwave cools the bulk liquid simultaneous with microwave heating. The bacteria apparently absorb microwave energy and are killed at bulk

temperatures insufficient to kill the bacteria normally. Further research is needed, and is planned, to eliminate the recycle and develop a once-through continuous process. Chemical and organoleptic analyses will determine if there is any thermal damage.

References

1 Rao, M. A. and Rizvi, S. S. H. *Engineering Properties of Foods*, 2nd Edn. New York, NY: Marcel Dekker, Inc, p. 401 (1995)

2 Mudgett, R. E. Electrical properties of foods in microwave processing. *Food Technology*, **36**, 109–115 (1982)

3 Goldblith, S. A. and Wang, D. I. C. Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. *Applied Microbiology*, **15**, 1371–1375 (1967)

4 Welt, B. A., Tong, C. H., Rossen, J. L. and Lund, D. B. Effect of microwave radiation on inactivation of *Clostridium sporogenes* (PA 3679) spores. *Applied and Environmental Microbiology*, **60**, 482–488 (1994)

5 Webb, S. J. and Dodds, D. D. Inhibition of bacterial cell growth by 136 gc microwaves. *Nature*, **218**, 374–375 (1968)

6 Webb, S. J. and Dodds, D. D. Absorption of microwaves by microorganisms. *Nature*, **222**, 1199–1200 (1969)

7 Culkin, K. A. and Fung, D. Y. C. Destruction of *Escherichia coli*, and *Salmonella typhimurium* in microwave-cooked soups. *Journal of Milk Food Technology*, **38**, 8–15 (1975)

8 Olsen, C. M. Microwaves inhibit bread mold. *Food Engineering*, **37**, 51–53 (1965)

9 Knorr, D., Geulen, M., Grahl, T. and Sitzmann, W. Food application of high electric field pulses. *Trends in Food Science & Technology*, **5** (3), 71–75 (1994)

10 Zimmermann, U., Pilwat, G. and Riemann, F. Dielectric breakdown of cell membranes. *Biophysical Journal*, **14**, 881–900 (1974)

11 Kinosita, K. Jr. and Tsong, T. Y. Formation and resealing of pores of controlled sizes in human erythrocyte membrane. *Nature*, **268**, 438–441 (1977)

12 Pothakamury, U. R., Barbosa-Canovas, G. V. and Swanson, B. G. Magnetic-field inactivation of microorganisms and generation of biological changes. *Food Technology*, **47** (12), 85–93 (1993)

13 Mertens, B. and Knorr, D. Developments of nonthermal processes for food preservation. *Food Technology*, **46** (5), 124–133 (1992)

14 ANON. Deactivation of microorganisms by an oscillating magnetic field. *World patent* 85/02094. San Diego, CA: Assigned to Maxwell Laboratories Inc (1985)

15 Levenspiel, O. *Chemical Reaction Engineering*, Ch. 9. New York, NY: John Wiley and Sons Inc (1967)